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# Collaborative Research: Mechanisms of Abiotic Immobilization of Nitrate in Temperate Forest Soils

David B. Dail

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**Final Report for Period:** 09/2005 - 08/2006**Submitted on:** 01/14/2007**Principal Investigator:** Dail, David B.**Award ID:** 0212675**Organization:** University of Maine

**Title:**  
 Collaborative Research: Mechanisms of Abiotic Immobilization of Nitrate in Temperate Forest Soils

### **Project Participants**

#### **Senior Personnel**

**Name:** Dail, David**Worked for more than 160 Hours:** Yes**Contribution to Project:**

#### **Post-doc**

#### **Graduate Student**

**Name:** Glessner, Michael**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Graduate masters obtained on this project; august 2005

#### **Undergraduate Student**

**Name:** Worcester, Megan**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Assited in this project for 2 summers (2004, 2005) as technical support

#### **Technician, Programmer**

#### **Other Participant**

#### **Research Experience for Undergraduates**

### **Organizational Partners**

#### **University of Arizona**

This was a collaborative project between the University of Maine, the University of Arizona and The Woods Hole Research Center

#### **Woods Hole Research Center**

This was a collaborative project between the University of Maine, the University of Arizona and The Woods Hole Research Center

### **Other Collaborators or Contacts**

Josh Schimmel UC Santa Barbara  
 Ben Colman UC Santa Barbara  
 Serita Frey Univ. of New Hampshire  
 Scott Ollinger Univ. of New Hampshire

## Activities and Findings

**Research and Education Activities:** (See PDF version submitted by PI at the end of the report)

**Findings:** (See PDF version submitted by PI at the end of the report)

### **Training and Development:**

During the term of the project one student (Michael Glessner) received his MS in Ecology and Environmental Sciences on this specific project. In addition, parts of the project were carried out by a summer intern (Ms. Megan Worcester) -a highschool math and science internship supported by the State of Maine (MERITS) program.

Both Mr. Glessner and Ms. Worcester delivered one or more talks at both local and national level meetings and also attended weekly lab meetings where aspects of the project were discussed

### **Outreach Activities:**

The project findings were presented to the Maine professional soil scientists group in 2003:

Maine Association for Professional Soil Scientists

<http://www.mapss.org/>

## Journal Publications

Thompson, A., J. Ruiz, O. A. Chadwick and J. Chorover, "Rayleigh fractionation of iron isotopes during pedogenesis.", Chem. Geol., vol. , ( ), p. . Accepted, "Rayleigh fractionation of iron isotopes during pedogenesis.", Chem. Geol., p. , vol. , ( ). Accepted,

Thompson, A., O. A. Chadwick, D. G. Rancourt and J. Chorover.

, "Iron-oxide crystallinity increases during soil redox oscillations", Geochim. Cosmochim. Acta, p. 1710, vol. 70, (2006). Published,

Glessner, M, Dail B., Davidson EA, and Chorover J., "Effects of long-term nitrogen additions on rapid nitrate immobilization in forest soils", Ecosystems, p. , vol. , ( ). in preparation,

Davidson, E.A., Dail, D.B., Chorover, J. Torres, P., Bulygina, E., "Iron interference of nitrate quantification in soil extracts: implications for studies of nitrogen immobilization", Soil Science Society of America Journal, p. , vol. , ( ). in preparation,

Villinski, J., E. A. Davidson, D. B. Dail and J. Chorover., "Iron reduction of nitrate in the presence of phenolic compounds", Soil Science Society of America Journal, p. , vol. , ( ). in preparation,

## Books or Other One-time Publications

Glessner, Dail, Davidson, Chorover, Villinski  
of Collection: , "Soil Science Society of America Annual Meeting Abstracts"  
, "Long-term nitrogen additions affect rapid nitrate immobilization by forest soils", (2004). Meeting abstract, Published  
Editor(s): bibl. Soil Science Society of America, Madison, Wisc., (2004). abstract Published  
Collection: Abstracts  
Bibliography: Soil Science Society of America, Madison, Wisc., (2004). abstract

M. Glessner, ""Effects of Long-term Nitrogen Additions on Rapid Nitrate Immobilization in Forest Soils"", (2005). Thesis, Published  
Editor(s): none

Collection: thesis

Bibliography: thesis; The University of Maine

M. Glessner, B. Dail, E. Davidson, J. Chorover, ""Factors Affecting Saturability of Nitrate Immobilization in Northern Forest Soils"", (2004). abstract, Published

Collection: HARVARD FOREST ECOLOGY SYMPOSIUM

Bibliography: Abstracts: HARVARD FOREST ECOLOGY SYMPOSIUM 2004

### Web/Internet Site

### Other Specific Products

### Contributions

#### **Contributions within Discipline:**

Based on our work from the preceding grant, our statement of the 'ferrous wheel hypothesis' (Davidson et al., Global Change Biology, 9:228-236,2003) has been widely cited as evidence for multiple elemental interactions in biogeochemical cycles, and for a previously unrecognized pathway for nitrate immobilization in soils. We are aware of submitted manuscripts from other researchers who cite our work as stimulation for their research. The mechanisms for nitrate retention in forest soils remain poorly understood.

Our hypothesized mechanism of nitrate immobilization by Fe(II) motivated several studies focusing on the rate, magnitude, and effects of iron redox cycling in forest soils. These studies involved the development of a well-controlled redox stat reactor that revealed significant changes in the form and reactivity

of Fe-bearing mineral phases during reduction-oxidation cycles (Thompson et al., 2006, Geochim. Cosmochim. Acta 70: 1710-1727). These studies suggest that significant changes in Fe mineral structure can occur over relatively short times in organic-rich forest soils, which has important implications for the Fe biogeochemistry field in general.

#### **Contributions to Other Disciplines:**

Based on informal conversations with colleagues from aquatic ecology, our 'ferrous wheel hypothesis' (Davidson et al., Global Change Biology, 9:228-236, 2003) for nitrate immobilization in forest soils has also influenced thinking about the fate of nitrate in aquatic ecosystems.

#### **Contributions to Human Resource Development:**

The project has involved high school students, undergraduate students (REU), graduate students, and a post-doc.

#### **Contributions to Resources for Research and Education:**

Davidson is serving on the scientific steering committee of the International Nitrogen Initiative ([www.initrogen.org](http://www.initrogen.org)), which promotes research and policy related to minimizing the negative impacts of excessive reactive nitrogen in the environment while maximizing the beneficial effects of nitrogen use for crop production.

Davidson also is a coPI for a Research Coordination Network (RCN) on denitrification, and has been actively involved in co-coordinating workshops, publications, and web resources development.

#### **Contributions Beyond Science and Engineering:**

As part of his work with the International Nitrogen Initiative, Davidson co-organized a workshop with the United Nations Environment Programme on Nitrogen Policy, held in Paris, March 2006. The workshop was designed to introduce policy makers to the issues of excess reactive nitrogen in the environment and to explore policy instruments at international levels that might address this issue. A workshop report is available at [http://www.whrc.org/policy/global\\_nitrogen.htm](http://www.whrc.org/policy/global_nitrogen.htm)

### Categories for which nothing is reported:

Any Web/Internet Site

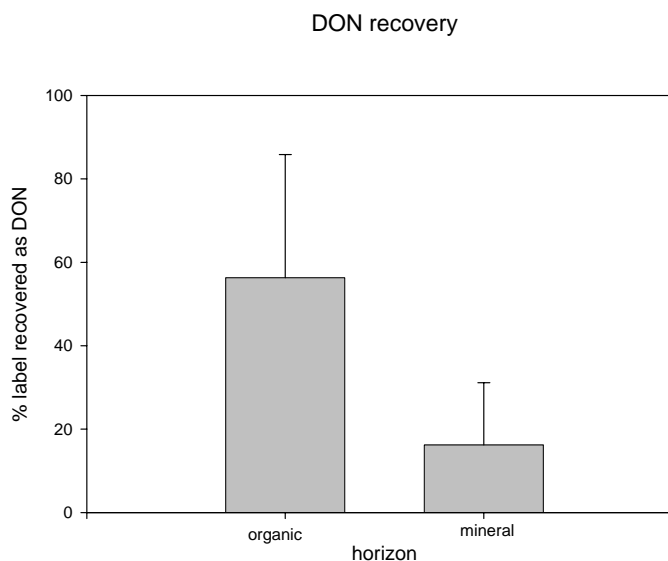
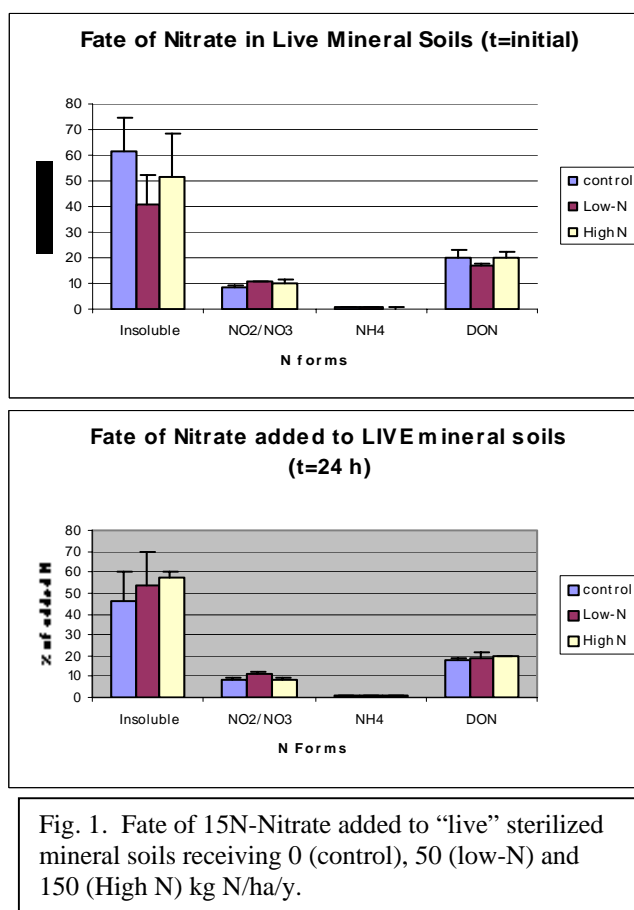
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## Collaborative Research: Mechanisms of abiotic nitrate immobilization in temperate forest soils

Eric A. Davidson (Woods Hole Research Center), Jon Chorover (University of Arizona) and D. Bryan Dail (University of Maine)

*Objective 1: Measure abiotic immobilization of nitrate in soils that have received experimental chronic N additions that may have altered soil constituents necessary for rapid N immobilization (D. Bryan Dail, University of Maine).*

Previously, we reported the findings for a  $^{15}\text{NO}_3^-$  addition to hardwood mineral soils. The major fate of added nitrate was an insoluble pool (Fig. 1), which includes soil solids and microbial cells that are collected by means of centrifugation. The second most important fraction was soluble organic nitrogen “DON”; about 20% of the added  $^{15}\text{N}$  ended up in this fraction. In all cases, less than 10% of the added nitrate remained in the form added. For organic horizon soils assayed in a similar manner, we confirmed that DON was the primary fate of added  $^{15}\text{NO}_3^-$  within the brief incubation period. A comparison of DON formed from nitrate in O-horizon vs. mineral soils is shown in Fig 2. Next, we compared the fate of  $^{15}\text{NO}_3^-$  in soils from the gradient of N fertilizer amendment histories at the Harvard Forest. At the time of soil sampling, these soils had received annual N loading of 50 (low-N) and 150 kg N/ha/yr (high-N) above ambient inputs for a period exceeding 12 years. Our expectation was that soil constituents necessary for the retention of  $^{15}\text{NO}_3^-$  whether by biotic or abiotic means, would have been substantially depleted by chronic fertilization and that DON formation would be highest in soils that had not been chronically fertilized (control > low-N > high-N). Results were consistent with this hypothesis but not statistically significant (Fig 3). We did not detect any association between the DON formation potential and the soil



content of soluble Fe and DOC; a relationship that we had previously observed to co-vary with DON formation in O horizon soils (Dail et al., 2001) and thus a critical component of our proposed model of abiotic DON formation in forest soils (Davidson et al., 2003). In sum, we observed a trend that was consistent with the expectation that chronically N fertilized soils would show a decrease in the capacity to immobilize additional  $\text{NO}_3^-$ . However, we could not conclude that concentrations of soil constituents (soluble Fe and DOC) thought to be important in abiotic immobilization mechanisms, explained these differences

*Objective 2: Identify the mechanisms of abiotic immobilization of nitrate in temperate forest soils (Jon Chorover, University of Arizona).*

**Detection of Nitrite.** Nitrite production from  $\text{Fe}^{2+}$  was detected in significant quantities in both the Cu(II) and the green rust (GRII) systems at pH 8 (Fig. 4). In the GRII system, the  $[\text{NO}_2^-]$  only reached 47  $\mu\text{M}$ , two orders of magnitude lower than  $[\text{NO}_3^-]_{\text{init}}$ . Therefore, in our system, conversion of  $\text{NO}_2^-$  to  $\text{NH}_4^+$  is the limiting step in the reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$ . A mass balance for this study indicates that dissolved inorganic nitrogen ( $[\text{NO}_3^-] + [\text{NO}_2^-] + [\text{NH}_4^+] = \text{DIN}$ ) is not statistically different from  $[\text{NO}_3^-]_{\text{init}}$ . In the presence of Cu(II), the production of  $\text{NO}_2^-$  was approximately 10% of the  $[\text{NO}_3^-]_{\text{init}}$ .

Pseudo-first order reaction rate constants were determined for the production of  $\text{NO}_2^-$  using the form

$$\frac{dC}{dt} = -\ln\left[1 - \frac{C}{C_{\text{max}}}\right]$$

where  $C_{\text{max}}$  is the maximum  $\text{NO}_2^-$  concentration of all the experiments (136  $\mu\text{M}$ ). The maximum concentration of all experiments was used to allow for inter-experimental comparison. Copper(II) is a much more effective catalyst for the reaction as evidenced by the fact that the reduction rate constant of  $\text{NO}_3^-$  and the formation rate constant of  $\text{NO}_2^-$  were three orders of magnitude greater in the presence of Cu(II) relative to GRII. Our hypothesis that  $\text{NO}_2^-$  is an intermediate species was verified and led us to test for nitration reactions in the presence of phenol and catechol.

**No Formation of Dissolved Organic Nitrogen.** Since  $\text{NO}_2^-$  was detected in the presence of both the Cu(II) and GRII catalysts, we then tested our hypothesis of nitrite incorporation into a phenolic compound through a nitration reaction. Results from stirred-reactor experiments with higher (1 mM)  $\text{NO}_2^-$  concentrations and various organic substrates at pH 4 resulted in nitration reactions (Davidson et al.,

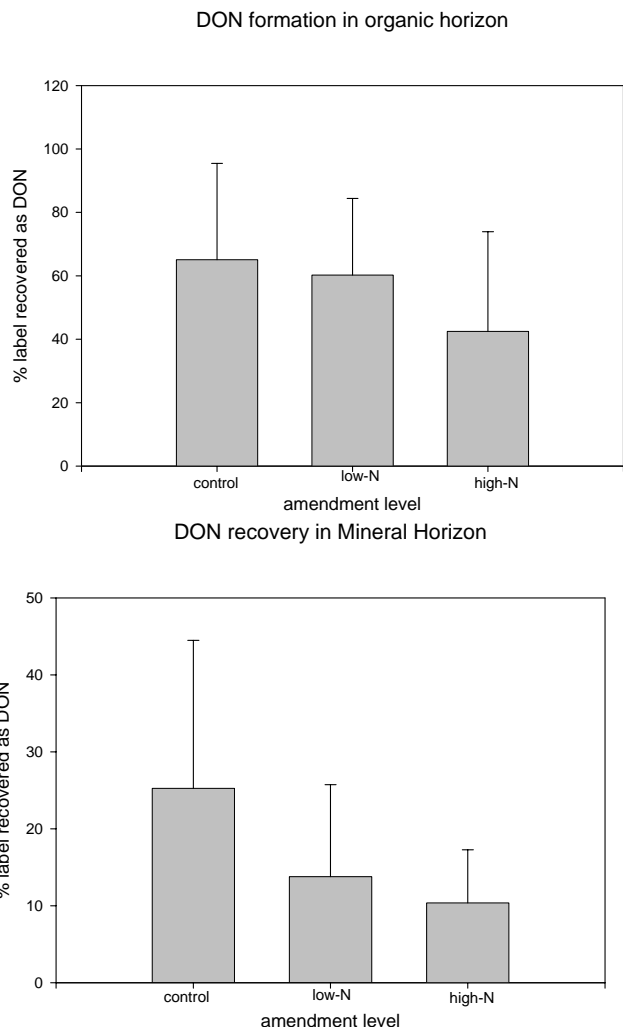


Fig 3. Dissolved organic N formation from an added  $^{15}\text{N}$   $\text{NO}_3^-$  label at time=0. Columns are the mean of 6 samples (3 each from two forest types) and error bars are 1 standard deviation of the mean.

2003). In those experiments, 4-nitrocatechol and 2-nitro 1-naphthol were detected in experiments with catechol and 1-naphthol, respectively, by HPLC–MS (Davidson et al., 2003). Figure 5 shows the time series of the  $\text{NO}_3^-$ , DIN and TDN concentrations in the presence of catechol at pH 8. In both systems, the TDN is statistically equal to the DIN, indicating no detectable quantities of organic nitrogen were formed. In the Cu(II) system, no significant quantities other species were noted in the HPCL–UV chromatograms. In the GRII system, the presence of *o*-quinone was detected, the oxidation product of catechol formed by Fe(III). No significant peaks attributable to a nitrogen-containing organic species were noted, however, consistent the TDN and DIN data.

**Effect of Catechol Concentration on the Reduction of  $\text{NO}_3^-$  by Fe(II).** The effect of catechol concentration (0, 1.1, and 8.7 mM) on the reduction of  $\text{NO}_3^-$  by Fe(II) was investigated in the GRII system (Fig. 6). The reduction of  $\text{NO}_3^-$  by Fe(II) in the GRII system with zero carbon present resulted in the reaction products  $\text{NH}_4^+$  (Fig. 6A) and magnetite. Fe(II) is the limiting reactant in this system; the pseudo-first order oxidation rate constant of  $\text{Fe(II)}_T$  at pH 8 was  $-8.3 \pm 1.8 \times 10^{-6} \text{ s}^{-1}$ .

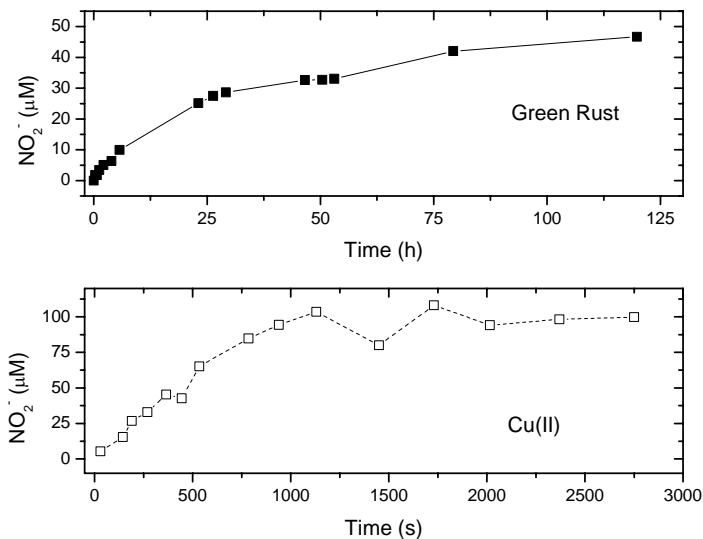


Figure 4. Production of nitrite during the reduction of nitrate by Fe(II) with zero carbon in green rust II (experiment 4) and Cu(II) (experiment 7) systems. Note the difference in the units of time.

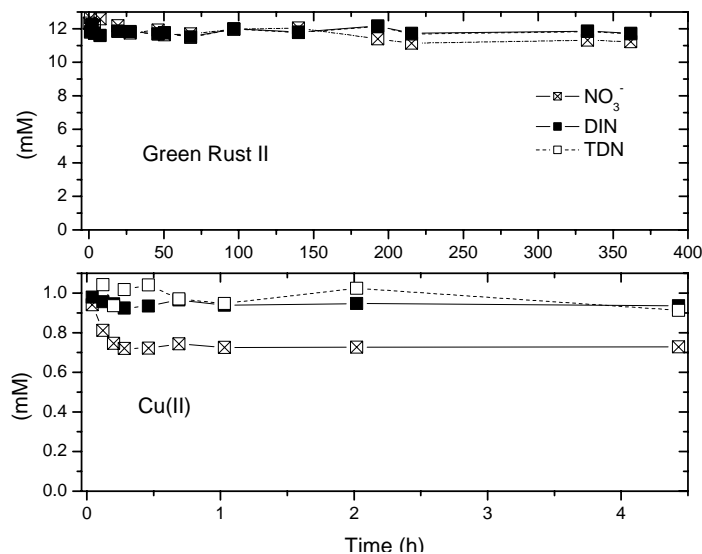


Figure 5. Time series of  $\text{NO}_3^-$ , DIN and TDN in the GRII (experiment 6C) and Cu(II) (experiment 8C) systems in the presence of catechol at pH 8.

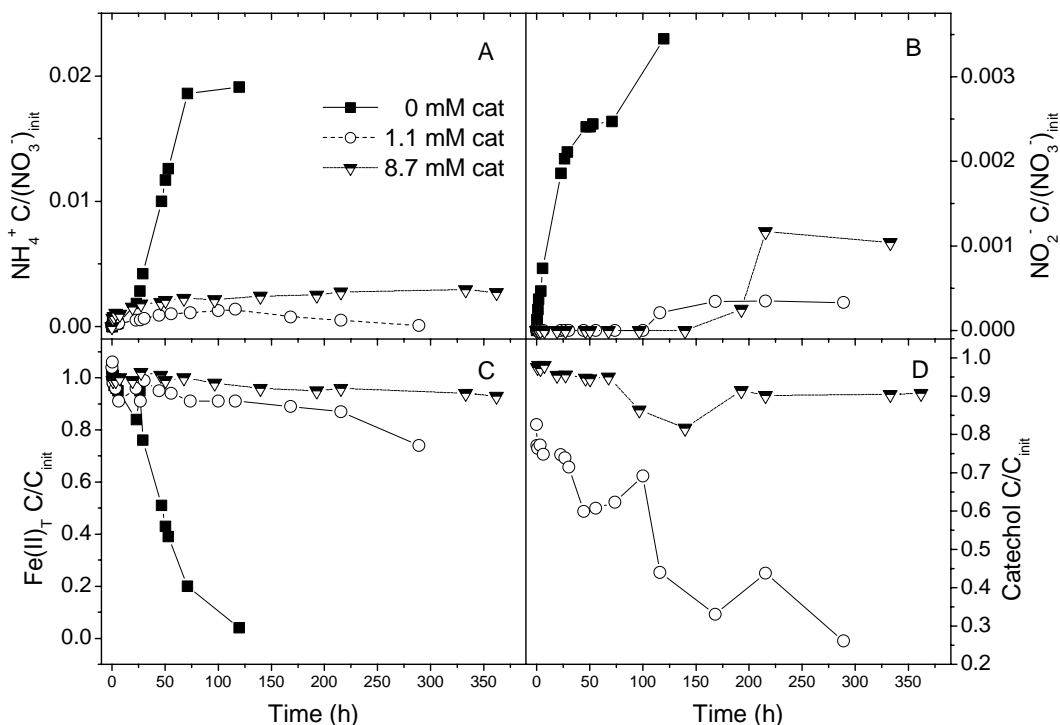


Figure 6. Concentration profiles showing the effect of catechol concentration on the GRII system at pH 8.

Pseudo-first order rate constants were determined for the production of  $\text{NH}_4^+$  for comparison across experiments using equation (1) with  $[\text{NH}_4^+]_{\text{max}} = 269 \mu\text{M}$ . The presence of catechol decreased the rate constants for the production of  $\text{NH}_4^+$  and the oxidation of  $\text{Fe(II)}_T$  by more than an order of magnitude (Fig. 6A). Since catechol is known to strongly complex with  $\text{Fe(III)(aq)}$  and sorb to mineral surfaces, we expect that catechol adsorbed to the surface  $\text{Fe(III)}$  of GRII. Indeed, aqueous phase measurements indicate that catechol adsorbs to the GRII; 9.4 and 17 mmole catechol  $\text{mole}^{-1} \text{Fe}_T(\text{s})$  for the 1.1 and 8.7 mM catechol experiments, respectively. Apparently, catechol adsorption reduces the surface reactivity of the GRII with respect to  $\text{NO}_3^-$  reduction, possibly by occupying  $\text{Fe(III)}$  surface sites that may otherwise have been occupied by reactive  $\text{Fe(II)}$ . In addition, XRD data indicated that the presence of catechol resulted in the conversion of the sulfate green rust (GRII) to a green rust I. The difference between GRII and GRI is the intercalating ion, with sulfate the intercalating ion in GRII, and  $\text{Cl}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{OH}^-$ , or  $\text{NO}_3^-$  in GRI.

Two results are of particular interest. First, the presence of catechol suppresses the accumulation of  $\text{NO}_2^-$  such that  $\text{NO}_2^-$  was not detected until 100–200 h (Fig. 6B). The lack of  $\text{NO}_2^-$  prior to this time would suggest that the catechol reduces the rate of reduction from  $\text{NO}_3^-$  to  $\text{NO}_2^-$  more than the reduction of  $\text{NO}_2^-$  to  $\text{NH}_4^+$  such that the conversion of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  is now the limiting step. The reason for the accumulation of  $\text{NO}_2^-$  after this early period would then suggest that some different mechanism was operative, hindering the conversion of  $\text{NO}_2^-$  to  $\text{NH}_4^+$ . Interestingly, the  $\text{NH}_4^+$  concentration either remains constant or decreases at the time when  $\text{NO}_2^-$  appears. However, the concentration profiles of the other species do not reveal any obvious trends that would explain these results (Fig. 6).

The second interesting result is that the total production of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  is greater in the presence of 8.7 mM catechol than in the presence of 1.1 mM catechol (Figs. 6A and 6B). One possible



explanation is that since catechol is capable of reducing Fe(III), more Fe(II) is available to reduce the  $\text{NO}_3^-$  (Fig. 5C). This reduction of Fe(III) is consistent with the apparent lower rate of Fe(II) oxidation in the presence of 8.7 mM catechol as compared to 1.1 mM catechol.

**Effect of Number of Phenolic Hydroxyl Groups on the Reaction.** The effect of the number of hydroxyl groups on the reduction of  $\text{NO}_3^-$  by Fe(II) was investigated in the Cu(II) catalyst system at pH 8 using phenol and catechol. Reaction products detected in the zero-carbon treatment include  $\text{NO}_2^-$  and  $\text{NH}_4^+$  (Fig. 7). In our experiment, with 1 mM Cu(II), within 45 min, when the reaction had slowed considerably, only 15% of the  $\text{NO}_3^-$  was converted into  $\text{NH}_4^+$  and 10% was converted into  $\text{NO}_2^-$ .

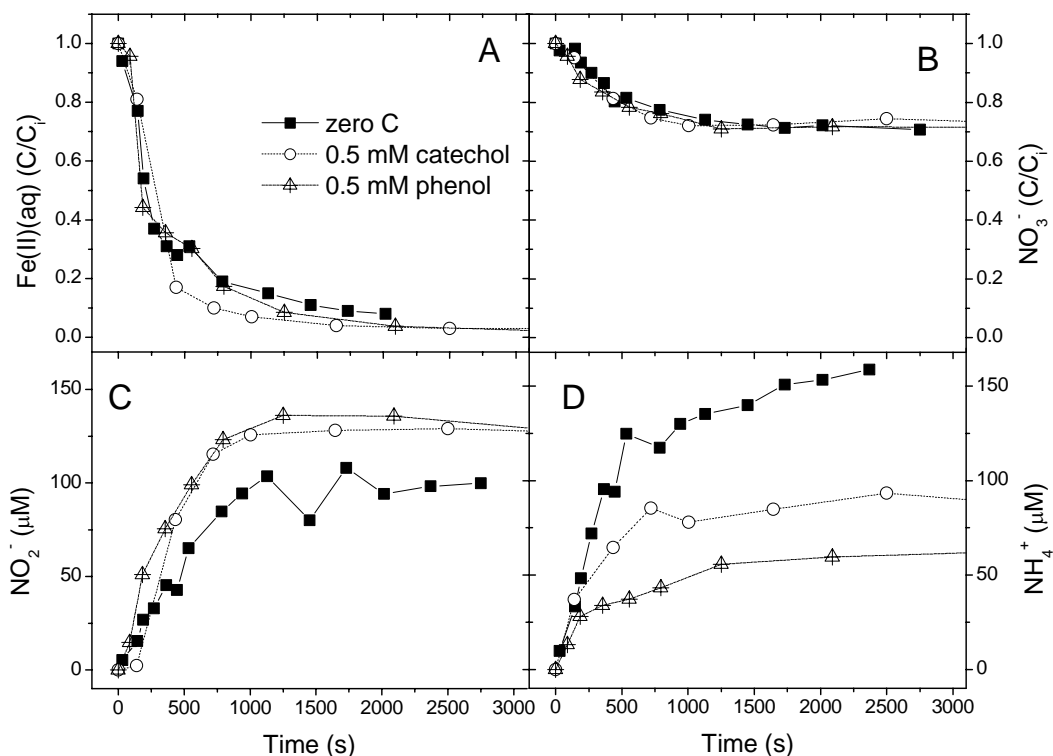


Figure 7. Effect of number of hydroxyl groups on the Cu(II)-catalyzed system at pH 8.

A poorly crystalline green rust II was the main solid phase product detected by XRD, with minor amounts of another solid, possible magnetite. The presence of a phenolic compound appeared to have little effect on the initial  $\text{NO}_3^-$  reduction and Fe(II) oxidation rate constants (Figs. 7A and 7B). However, presence of a phenolic compound did change the relative rates of the formation of  $\text{NO}_2^-$  and  $\text{NH}_4^+$  (Fig. 7C), decreasing the initial pseudo-first order rate constant of  $\text{NH}_4^+$  production by half while increasing the apparent rate constant of  $\text{NO}_2^-$  production by a factor of two. Since the rate of  $\text{NO}_3^-$  reduction is statistically the same, the effect of the phenolic compound is to inhibit the reduction of  $\text{NO}_2^-$  to  $\text{NH}_4^+$ .

The presence of catechol had different effects in the two systems, suppressing the formation of N-reaction products in the GRII system, and merely decreasing the conversion rate of  $\text{NO}_2^-$  to  $\text{NH}_4^+$  in the Cu(II) system. To more fully illustrate this, pseudo-first order reaction rate constants were determined for the combined number of electrons transferred from  $\text{NO}_3^-$  to  $\text{NO}_2^-$  (2) and  $\text{NH}_4^+$  using equation (1) and assuming no gaseous N products were formed. The rate constant of  $e^-$  transfer decreased by a factor of

two in the Cu(II) system, but by more than an order of magnitude in the GRII system. A probable explanation is that in the GRII system, the catechol to Fe(II)(aq) ratio is much higher than in the Cu(II) system. Therefore, competition for catalytic surface sites is much greater in the GRII system than in the Cu(II) system.

The number of phenolic hydroxyl groups affects the crystal structure of the iron precipitate. With 0.5 mM phenol present, the GRII formed is more poorly crystalline than in the presence of no carbon. With 2 adjacent hydroxyl groups (0.5 mM catechol), the green rust crystal formation is completely disrupted, forming an unknown, poorly crystalline, orange–brown precipitate. This result is consistent with published results showing that organic compounds which can complex Fe(III) disrupt the formation of iron minerals.

*Objective 3: To investigate the bioavailability and ultimate fate of dissolved organic N produced by either microbial or abiotic immobilization of nitrate (Eric Davidson, The Woods Hole Research Center).*

Nitrate: The BioRad AG 1-X8 anion exchange resin effectively removed nitrate in solution to below detection limits (<0.1 mg N/L). However, despite the fact that nitrate was below detection limits using our Lachat autoanalyzer, diffusions of the extracts for  $^{15}\text{N}$  analyses revealed a trace amount of the originally added  $^{15}\text{NO}_3^-$  label. This trace contamination of original label undermines this approach for isolating a labeled DON fraction in which all of the label is unequivocally in the DON form. Hence, the SR7 resins, which are marketed specifically for their high affinity to nitrate, were also tested. Again, the SR7 resins removed nitrate to below detection limits using our Lachat autoanalyzer. At the time of writing this report, we are still preparing diffusions of these extracts to send off for  $^{15}\text{N}$  analysis. Although our project and funding have ended, we are continuing to pursue these analyses without funding.

Ammonium: The BioRad AG 50W-X8 cation exchange resin reduced ammonium concentrations from 2 – 3 mg  $\text{NH}_4^+$ -N/L to <0.2 mg  $\text{NH}_4^+$ -N/L. However, the resins appeared to gradually lose their effectiveness with increased use. Regenerating the resins with LiOH solution only partially recharged the effectiveness. To use these resins to remove ammonium from solution, it will be necessary to periodically test their effectiveness. We also suspect that ammonium continues to be mineralized from DON as soil extracts are stored under alkaline conditions. Hence, extracts must be acidified before storage. However,  $^{15}\text{N}$  analysis of diffusion of the extracts have revealed that no label was found in the  $\text{NH}_4^+$  pool, therefore, removing this pool from the extract is not as crucial as we originally thought for obtaining a DON product with the label unequivocally in the DON form.

Dissolved Organic Nitrogen (DON): The DON fractions obtained after removal of ammonium and nitrate using the BioRad resins was not significantly enriched in  $^{15}\text{N}$  beyond what might be attributed to a trace level of  $^{15}\text{NO}_3^-$  label remaining in solution. Hence, we have not yet succeeded in obtaining a DON fraction that is unequivocally labeled with  $^{15}\text{N}$ . Our continued work with the SR7 resins, which have a greater affinity specifically for nitrate, holds promise for eventually reaching our objective.

Although it has been somewhat frustrating that we have not yet achieved our goal of obtaining an unequivocally labeled  $\text{DO}^{15}\text{N}$  fraction that can be used for subsequent studies of bioavailability of DON, we have made progress in carefully analyzing the pros and cons and potential artifacts of several methodologies. Our ongoing work with a new type of resin with high affinity for nitrate holds promise that we may yet achieve our objective through painstaking, careful procedures.

Potential for iron interference with the determination of nitrate (Dail and Davidson):

We were made aware that there was a potential for soluble Fe to interfere with the quantification of nitrate, and that the typical method of alleviating this interference was not particularly effective in soils with high Fe content (unpublished results of another research group). We tested this potential interference at both the University of Maine (Dail) and at the Woods Hole Research Center (Davidson). First, we generated filtered solutions of varying concentrations of Fe in both the ferric and ferrous sulfate salts at pH 6. We spiked those solutions a  $\text{NO}_3^-$  solution to give a final concentration of 2.4 mg N/L  $\text{NO}_3^-$  and assayed them as soon as possible using the  $\text{NH}_4\text{Cl}/\text{EDTA}$  method. We observed that at high Fe content, ( $>5$  mg Fe/l) there was a potential for both ferrous and ferric salts to interfere with  $\text{NO}_3^-$  determination (Fig 8).

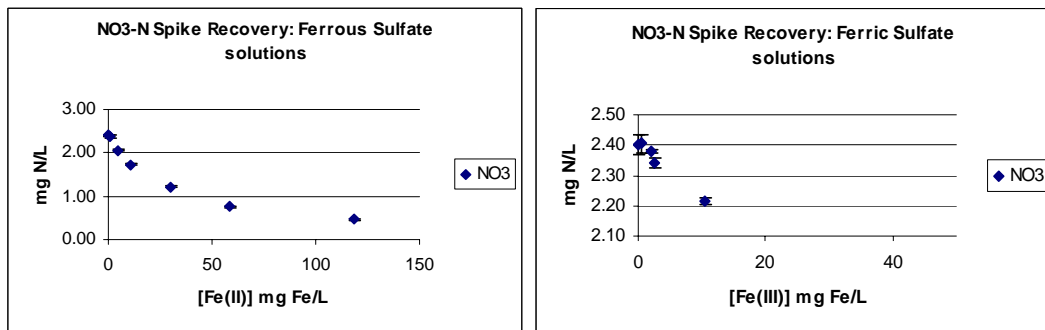


Fig 8. Recovery of a  $\text{NO}_3^-$  spike in ferric and ferrous sulfate iron solutions. Fe standards at pH 6 were filtered prior to analysis; however Fe probably continued to fall out of solution during analysis. Fe contents were determined on separate samples within 1 day of the nitrate analysis. Datapoints are the mean of 3 determinations with error bars representing 1 standard deviation of the mean (Dail).

We then tested soil extracts from several mineral soils and the O horizon from Harvard Forest under different fertilization regimes and treatments. Autoclaving increased the release of soluble Fe and was suspected of creating problems with  $\text{NO}_3^-$  determination. While these results demonstrated that appreciable native soluble Fe contents existed, spikes of soils extracts with nitrate were completely recoverable under the conditions of our extraction procedure (Table 1).

**Table 1.** Investigation of Fe interference with water extracted soils from Harvard Forest in Dail's lab at the University of Maine. Soluble Fe and nitrate-N reported in soil extract as-is or with a nitrate spike to increase the nitrate concentration by 2.41mg N/L. High N plots soil have received 150 kg N for over a decade.

Soil	Fe(solb) (mg Fe/l)	$\text{NO}_3^-$ (native) (mg N/l)	$\text{NO}_3^-$ (native+spike) (mg N/l)	spike recovery (%)
<b>Control plot soils</b>				
O horizon				
Live	1.10	0.68	3.12	101
Sterile	1.01	0.68	3.14	101
Mineral horizon				
Live	0.21	2.39	4.89	102
Sterile	0.53	2.28	4.78	102
<b>High-N plot soils</b>				
O horizon				
Live	0.76	12.12	14.59	100
Sterile	2.22	11.82	14.53	101
Mineral horizon				
Live	0.74	3.53	6.39	101
Sterile	3.43	3.92	5.89	99

Table 2. Standard addition of nitrite or nitrate to extracts of sterile and live soils and to standards in dH<sub>2</sub>O in Davidson's WHRC lab.

NO <sub>2</sub> + NO <sub>3</sub> (mg N/L)				
Sample	Rep	Background	Background + 2ppm NO <sub>3</sub>	Background + 2ppm NO <sub>2</sub>
0.4ppm NO <sub>2</sub> std	A	0.44		2.62
0.4ppm NO <sub>2</sub> std	B	0.45		2.65
0.4ppm NO <sub>2</sub> std	C	0.45		2.65
Sterile	A	0.51	2.54	2.65
Sterile	B	0.73	2.64	2.87
Sterile	C	0.36	2.68	2.48
Live	A	0.16	2.35	2.01
Live	B	0.13	2.19	2.48
Live	C	0.19	2.48	2.44
0.4ppm NO <sub>3</sub> std	A	0.38	2.59	
0.4ppm NO <sub>3</sub> std	B	0.36	2.57	
0.4ppm NO <sub>3</sub> std	C	0.36	2.54	

To further investigate the mechanism of reported iron interference of nitrate quantification using the NH<sub>4</sub>Cl-EDTA buffer method, we conducted experiments to test the effect of varying iron concentrations on quantification of nitrite standards and of both nitrite and nitrate standards following persulfate digests for total dissolve nitrogen (TDN) analyses. The addition of soluble iron reduced the efficacy of both nitrate and nitrite quantification (Fig 9). Therefore, it is likely that high concentrations of soluble iron interfere with the colorimetric analysis of nitrite rather than the reductive step of nitrate to nitrite. Moreover, this happens even when the iron is chelated by EDTA -- a chelator added specifically to remove Fe interference in the method. In contrast, the problem does not occur in persulfate digests, which suggests that oxidation of the iron during the persulfate digests removes the interference with nitrate quantification. Although this interference is obvious when soluble iron is added at relatively high concentrations, it may not be a problem in most soil extracts. As reported in Table 1, we measured soluble iron concentrations of <4 mg Fe L<sup>-1</sup> in our soil extracts. We again repeated the standard addition experiments conducted by Dail in Table 1, but this time included standard additions of nitrite in Davidson's lab. The results shown in the Table 2 demonstrate that the recovery of both added

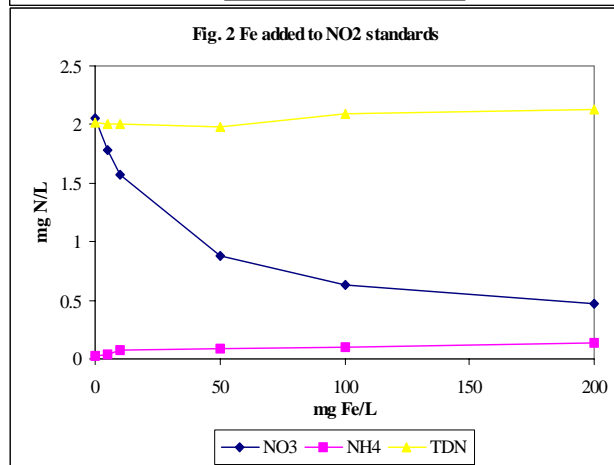
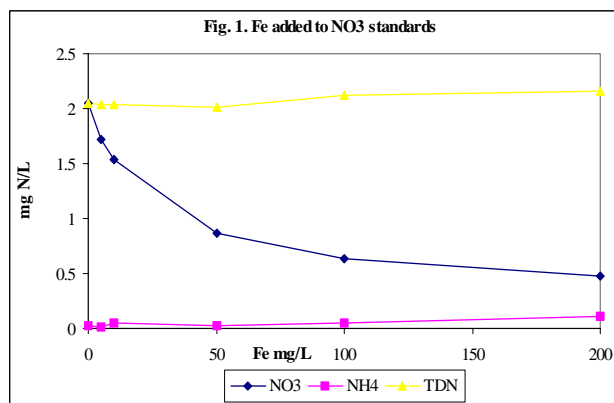


Figure 9. Effects of soluble iron concentrations on quantification of ammonium, nitrate, and total dissolved N (TDN) in standards of either nitrate (top panel) or nitrite (lower panel) in dH<sub>2</sub>O (Davidson).

nitrate and nitrite was near 100% in both standard solutions and Harvard Forest soil extracts to which standards were added. Hence we conclude, that, while iron interference may an important consideration that should be checked where soluble iron concentrations might be high, it has not significantly affected our results.

## Summary

*Objective 1: Measure abiotic immobilization of nitrate in soils that have received experimental chronic N additions that may have altered soil constituents necessary for rapid N immobilization (D. Bryan Dail, University of Maine).*

Using soils from a chronic-N experiment at the Harvard Forest, we reconfirmed previous results that  $^{15}\text{NO}_3^-$  was abiotically retained in the soil, primarily as DON. There was a trend of lower  $^{15}\text{NO}_3^-$  conversion to DON in the plots that had chronically received N additions during the last decade, compared to the control soils (control > low-N > high-N), but the effect of previous N treatment was not statistically significant. Hence, the abiotic immobilization process was, at most, only partially saturated by chronic N additions.

*Objective 2: Identify the mechanisms of abiotic immobilization of nitrate in temperate forest soils (Jon Chorover, University of Arizona).*

The presence of catechol reduced the rate of nitrate reduction by Fe(II) in the presence of the green rust catalyst at pH 8 by an order of magnitude. While nitrite was detected in quantities up to 270  $\mu\text{M}$  in the absence of catechol, no organic nitrogen products were detected when catechol was present. The presence of a phenolic compound did not decrease the apparent conversion of  $\text{NO}_3^-$  in the Cu(II)-catalyzed system at pH 8, however, the phenol did decrease the conversion of  $\text{NO}_2^-$  to  $\text{NH}_4^+$ , with catechol decreasing the conversion rate more than phenol. As in the GRII-catalyzed system, no organic nitrogen reaction products were detected. Decreasing the pH to 5 effectively stopped the reaction. This evidence suggests that an additional mechanism of abiotic immobilization of nitrate that we had not considered in the original formulation of the ferrous wheel hypothesis is the abiotic reduction of nitrate to ammonium by soil minerals and subsequent reactions of ammonium with soil organic matter. This modification of the ferrous wheel hypothesis merits further consideration and research.

*Objective 3: To investigate the bioavailability and ultimate fate of dissolved organic N produced by either microbial or abiotic immobilization of nitrate (Eric Davidson, The Woods Hole Research Center).*

We have not yet achieved an unequivocally labeled  $\text{DO}^{15}\text{N}$  fraction that can be used for subsequent studies of bioavailability of DON, but we have made progress in carefully analyzing the pros and cons and potential artifacts of several methodologies. Our ongoing work with a new type of resin with high affinity for nitrate holds promise that we may yet achieve our objective.

*Additional studies on iron interference of nitrate quantification:*

Our experiments have shown that the most likely step in the analytical procedure that may be affected by soluble iron is the colorimetric reaction with nitrite. While we were able to reproduce reports of iron interference with nitrate quantification when added soluble iron concentrations are relatively high, we conclude that this interference has not significantly affected our results at iron concentrations typically

encountered in our soil extracts. Hence, our previous and new estimates of abiotic immobilization of nitrate appear to be robust.

The ferrous wheel hypothesis of abiotic immobilization of remains a viable, but still controversial mechanism to help explain the frequently observed high retentive capacities of forest soils for nitrate from atmospheric deposition and from experimental additions. From this research project we have learned that the abiotic nitrate immobilization mechanism did not appear to be saturated by chronic-N additions, and that it may be more complex than previously thought, including a possible role for reduction of nitrate to ammonium.

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## **Collaborative Research: Mechanisms of abiotic nitrate immobilization in temperate forest soils**

Eric A. Davidson (Woods Hole Research Center), Jon Chorover (University of Arizona) and  
D. Bryan Dail (University of Maine)

### **Project Rationale**

Atmospheric deposition of nitrogen to temperate forests has increased in recent decades as a result of increased combustion of fossil fuels. Because N often limits rates of plant growth, increased N inputs could affect several ecosystem processes, including carbon sequestration by forests. However, most of this additional N is retained in soil organic matter rather than being taken up by plants, where it could promote greater carbon storage in woody biomass. The explanation for N retention in forest soils is likely related to the mechanisms of immobilization, which remain poorly understood. Recent evidence that *abiotic* immobilization of N may be an important process challenges a widely held view that microbial processes are the dominant pathways for N immobilization in soil. In a previous project, we described a process of abiotic nitrate ( $\text{NO}_3^-$ ) immobilization into dissolved organic nitrogen (DON). Abiotic immobilization of  $\text{NO}_3^-$  is particularly perplexing because the thermodynamics of  $\text{NO}_3^-$  reduction in soils are not favorable under oxic conditions. The mechanism of immobilization may also affect the fate of DON. Whereas turnover of soil microbial biomass releases biotically immobilized N into inorganic-N that can be taken up by plants, we know little about the fate of DON and nothing of whether the DON formed by abiotic immobilization of  $\text{NO}_3^-$  is eventually remineralized into a plant-available form.

### **Overview of Approach**

We took advantage of an ongoing N addition experiments in the Harvard Forest of central Massachusetts. Our first objective was to measure immobilization of nitrate in these forest soils that have received N additions experimentally. We sought to determine whether N addition has changed the capacity of soils to assimilate nitrate, either biotically or abiotically. A previously developed protocol for measuring abiotic immobilization of nitrate was being applied to a variety of soils and litters under experimentally controlled incubation conditions. Our working hypotheses, dubbed the “ferrous wheel hypothesis” by Davidson et al. (2003), is that iron ( $\text{Fe}^{\text{II}}$ ) in anaerobic microsites can reduce  $\text{NO}_3^-$  to nitrite ( $\text{NO}_2^-$ ) and that  $\text{NO}_2^-$  reacts with DOC to form DON. The  $\text{Fe}^{\text{II}}$  is then regenerated by reduction of  $\text{Fe}^{\text{III}}$ -bearing soil minerals coupled to oxidation of DOC. This mechanism could be saturated if nutrient imbalances due to high N inputs cause a decline in primary productivity and decreased input of DOC to soil. Our second objective was to test this hypothesis in several laboratory experiments, where the concentrations and combinations of hypothesized reactants were varied systematically to determine how they affect rates of abiotic immobilization of nitrate. After demonstrating that DON is formed from nitrate and elucidating the mechanisms, the next logical step is to address the fate of DON. Our third objective was to develop a realistic  $^{15}\text{N}$ -labelled DON product that can be used to study the bioavailability of this important form of N in terrestrial ecosystems.

### **Project organization**

This collaborative project was carried out by the three principal investigators, with each in charge of one of the three objectives summarized above. We present here an integrated report of all three subprojects. This combined report is being submitted by each PI for his respective grant.

*Objective 1: Measure abiotic immobilization of nitrate in soils that have received experimental chronic N additions that may have altered soil constituents necessary for rapid N immobilization. (D. Bryan Dail, University of Maine).*

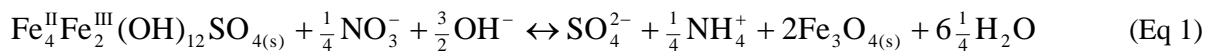
Soils were collected in October 2002 and again in June of 2004 from the “chronic-N” experiment initiated at the Harvard Forest in 1988 (Aber et al., 1989). Mineral horizon soils (October 2002) from two stand types; a pine plantation and a native deciduous hardwood forest were collected and assayed for their retention of an  $^{15}\text{N}$  nitrate label. Soils from these stands have been receiving amendments of 0, 50, and  $150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , as  $\text{NH}_4\text{NO}_3$ , divided into six doses applied to the forest floor for over 14 years. We used an  $^{15}\text{N}$  tracer (99%  $\text{Na}^{15}\text{NO}_3$ ) to investigate whether the 14 years of fertilization will have affected the potential for abiotic formation of dissolved organic nitrogen, an observation that accounted for 30-50% of nitrate disappearance from non-fertilized soil from this site (Berntson and Aber, 2000; Dail et al., 2001).

We applied the methodology of Dail et al. (2001) to the hardwood and pine plantation mineral soils from experimental plots at the Harvard Forest to trace the fate of nitrate in soils in short term incubations; sample removals occurred at 15 minutes (hereafter  $t=\text{initial}$ ) and 24 hours later ( $t=24 \text{ h}$ ). Soils were pre-treated by either bringing a frozen soil to room temperature (live soil) or autoclaving the soil (sterilized soil) before adding  $^{15}\text{N}$  tracer. The mineral soils were assayed in late 2002 and early 2003; and because of the cumbersome nature of the experimental design (both in sample number and time needed to run the assay to completion), we opted to get fresh samples in summer 2004 in order to perform this assay on the O horizon; that component of the forest floor where we had previously measured rapid interaction with added nitrate in previous work (Dail et al., 2001).

In September 2005 we were made aware of a potential metal interference (Fe) with the determination of  $\text{NO}_3$  using the  $\text{NH}_4\text{Cl}$ -EDTA method (1M EDTA is reported to reduce or eliminate Fe interference in most methods reviewed by us) (unpublished data). The consequence of an interference in solutions with high soluble Fe would be an underestimation of  $\text{NO}_3$  content, and because DON is determined “by difference” ( $\text{DON}=\text{TDN}-\text{NO}_3+\text{NH}_4$ ) an overestimation of the content of DON; a critical determination for this study. In late 2005 and early 2006 we focused our efforts on investigating the importance of this potential problem under the experimental conditions used in past work.

*Objective 2: Identify the mechanisms of abiotic immobilization of nitrate in temperate forest soils (Jon Chorover, University of Arizona).*

To probe possible mechanisms, “soil component” experiments focusing on well-defined ferrous iron systems were chosen as the starting point. Recent research has shown that the ferrous-ferric mineral, *green rust*, may be an important component of forest and hydromorphic soils (Génin et al., 1998). In addition, green rust has been shown to be an effective reductant of nitrate (Hansen and Koch, 1998). The sulfate form of green rust ( $\text{GR}_{\text{SO}_4}$ ) was therefore chosen as a candidate for the “soil component” of the  $\text{Fe(II):NO}_3^-:\text{DOM}$  system. The reaction by which nitrate is reduced by  $\text{GR}_{\text{SO}_4}$  is:





Nitrogen may then be incorporated into DOM when nitrate is reduced to 1) nitrite (a possible intermediate in the reaction above) or 2) ammonium.

Several preparation methods were investigated to determine which method is best for reproducibly producing  $\text{GR}_{\text{SO}_4}$ . The method by Refait and Génin (2003) was chosen, and the identity of the precipitate was confirmed using x-ray diffraction (XRD) (Figure 1). As  $\text{GR}_{\text{SO}_4}$  is unstable in the presence of  $\text{O}_2$ , an anaerobic glove box was set-up for all experimental procedures. The experiments are performed in batch-mode at constant temperature and pH.

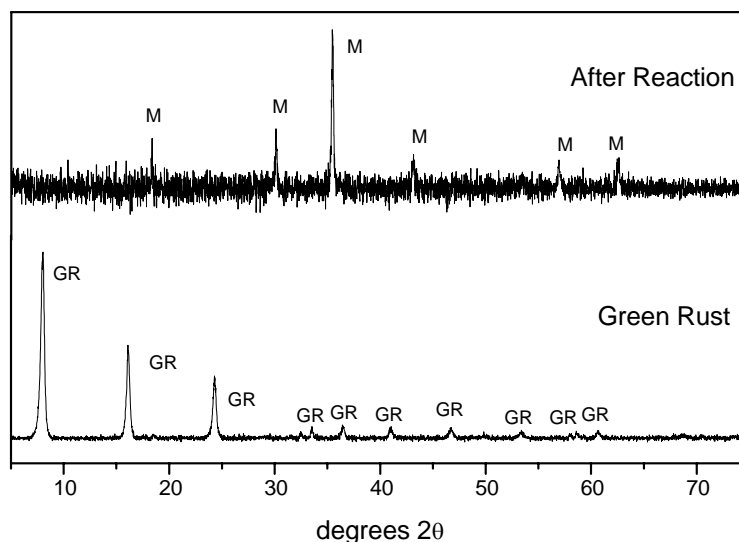


Figure 1. X-ray diffraction patterns of  $\text{GR}_{\text{SO}_4}$  prepared by the method of Refait et al. (2003) before and after 76 h reaction with nitrate indicate that all the  $\text{GR}_{\text{SO}_4}$  has been converted to magnetite (GR = green rust; M = magnetite).

A series of laboratory experiments were performed to investigate the effect of simple phenolic compounds on the reduction of nitrate by  $\text{Fe}^{\text{II}}$  in green rust. We investigated the effects of two phenolic compounds, phenol and catechol, on the reduction of nitrate by  $\text{Fe}^{\text{II}}$  in the presence of two different catalysts,  $\text{Cu}^{\text{II}}$  and sulfate green rust. The two chosen phenolic compounds present very different qualities. Catechol, with two adjacent hydroxyl groups, forms strong, bidentate complexes with  $\text{Fe}^{\text{III}}(\text{aq})$ , and under the right conditions, can be oxidized by  $\text{Fe}^{\text{III}}$ . The standard reduction potential for  $\text{Fe}^{\text{III}}$  is 0.771 V and for o-quinone to catechol is 0.792 V. Phenol, on the other hand, with a single hydroxyl group, is a very weak metal complexant. It was found to not sorb to  $\text{Fe}^{\text{III}}$  minerals and is a poor reductant, with a standard reduction potential of 1.34 V.

All experiments were performed in a glove box with a 3%  $\text{H}_2$  – 97%  $\text{N}_2$  gas mixture to exclude oxygen. DI water was deoxygenated by bubbling with  $\text{N}_2$  for 30 min prior to placement in the glove box. The dissolved oxygen (DO) level ( $< 10 \mu\text{g L}^{-1}$ ) was confirmed using Hach Low- $\text{O}_2$  AccuVac ampules.

**Kinetic Experiments.** The constantly-stirred reactor experiments were performed at 25° C in a jacketed beaker. The pH was kept constant using a 751 GPD Titrino (Metrohm Corp) with an ~0.1 M NaOH solution. Two catalysts were used in these experiments:  $\text{Cu}^{\text{II}}$  and green rust II ( $\text{GR}^{\text{II}}$ ).

For the  $\text{GR}^{\text{II}}$  system, sulfate  $\text{GR}^{\text{II}}$  was synthesized using the procedure of (Refait et al., 2003). A 0.2 M total Fe ( $\text{Fe}_{\text{T}}$ ) was made with  $\text{Fe}^{\text{II}}$ - and  $\text{Fe}^{\text{III}}$ -sulfate salts with an  $\text{Fe}^{\text{II}}:\text{Fe}^{\text{III}}$  ratio of 3:1. Four hundred mL of this solution was mixed with 0.4 L of 0.3 M NaOH. The solution was aged in the glove box for 24 h. The precipitate was identified as  $\text{GR}^{\text{II}}$  using powder X-ray diffraction (XRD). The X-ray diffraction analyses were performed at 30 keV with  $\text{Cu}_{2\alpha}$  radiation on a Philips X'Pert Pro MPD powder and thin-film X-ray diffractometer with either wet solid or freeze-dried solid coated with glycerin to prevent oxidation. The  $\text{GR}^{\text{II}}$  was washed three times with DI water and centrifuged, and then added to ~200 mL of 1 mM  $\text{FeSO}_4$  solution. The pH was adjusted to 8 and a sample was collected. For the 0.5 mM catechol

and phenol experiments, an aliquot of freshly made 0.1 M catechol solution was added, the pH was adjusted, and a sample was collected. The reaction was started by adding an appropriate volume of a stock 286 mM  $\text{NaNO}_3$  solution to result in an  $[\text{NO}_3^-]_{\text{init}}$  of  $\sim 13$  mM.

For the Cu system, a 1 mM solution of each  $\text{NaNO}_3$  and  $\text{CuCl}_2$  was equilibrated at the desired pH (5 or 8). Analysis indicated that the Cu- $\text{NO}_3$  solution was non-reactive. The reaction was started by adding an aliquot of  $\text{FeSO}_4$  resulting in a 10 mM final  $\text{Fe}^{\text{II}}(\text{aq})$  concentration. For the 0.5 mM catechol and phenol experiments, an aliquot of freshly made 0.1 M “phenol” solution was added to the reaction vessel at the same time as the  $\text{Fe}^{\text{II}}$ .

Samples up to 5 mL were fractionated for the various analytes measured. The procedure of (Koch et al., 1997) was used to determine the total  $\text{Fe}^{\text{II}}$  and  $\text{Fe}_\text{T}$  in the system, with  $\text{Fe}^{\text{III}}$  determined as the difference. One portion of the suspension was diluted in 25 mL of 10 mM HCl without filtration to dissolve amorphous iron hydroxides and/or green rust formed during the reaction to determine total available  $\text{Fe}^{\text{II}}$  and total dissolvable  $\text{Fe}_\text{T}$ . After 5 min, 2 mL of this “total iron” sample was passed through a 0.2  $\mu\text{m}$  filter into 10 mL of 1 mM HCl, with the first 0.5 mL filtered to waste. The rest of the suspension sample was passed through a 0.2  $\mu\text{m}$  filter, with the first 0.5 mL filtered to waste. Nitrate, nitrite, and sulfate (detection limit of  $\sim 1$   $\mu\text{M}$  for all analytes) were analyzed within 5 h by ion chromatography using a DX500 system with an AS11 separator column (Dionex Corp.). Phenol, catechol and organic reaction products were analyzed within 5 h by HPLC-UV/Vis using a 600 E pump and a 996 photoelectric diode array (PDA) UV/Vis spectrophotometer (Waters Corp). The organic reactants and products were separated using a 20 cm  $\times$  4 mm ID Aquasil C18 column (Thermo-Hypersil-Keystone) at 0.4 mL  $\text{min}^{-1}$  using a gradient run ramping from 100% DI to 100% methanol over 15 min, holding at 100% methanol for 5 min, and returning to 100% DI over the next 5 min. The  $\text{Fe}^{\text{II}}$  and  $\text{Fe}_\text{T}$  measurements (with  $\text{Fe}^{\text{III}}$  determined by difference) were made with the ferrozine method, using a PC2150 UV/Vis spectrophotometer (Shimadzu Corp). For ammonium analyses, 0.5 mL was placed in a amber glass vial, and 10  $\mu\text{L}$  of concentrated HCl was added as a preservative and sealed. The  $\text{NH}_4^+$  was analyzed using the same Dionex IC system with a CS14 separator column; detection limit  $\sim 1$   $\mu\text{M}$ . Total organic nitrogen (TOC) and total dissolved nitrogen (TDN) were analyzed on Shimadzu TOC-VCHS and TNM-1 modules, respectively, using an ASI-V autosampler. Any remaining filtered, undiluted sample was frozen for future use. The estimated error on all measurements was less than or equal to  $\pm 5\%$  based on calibration standards.

*Objective 3: To investigate the bioavailability and ultimate fate of dissolved organic N produced by either microbial or abiotic immobilization of nitrate (Eric Davidson, The Woods Hole Research Center).*

Studying the fate of DON would be facilitated by the development of a  $^{15}\text{N}$  label in a realistic soil DON product. During the first year of the project, we tested incubation conditions that would permit nitrate immobilization to DON during 24 h, followed by conditions that would minimize the remaining nitrate. Although denitrification during incubation reduced nitrate concentrations to near detection limits, significant amounts of ammonium were released during anaerobic incubation. Therefore, this approach was successful in removing only one of the two dissolved inorganic-N (DIN) components of the soil solution. Our goal was to produce an  $\text{N}^{15}$ -labeled DON solution free of DIN.

During the second year, we tested Bio-Rad cation and anion exchange resins to remove DIN from soil extracts. During the third year, we tested the SR7 nitrate-specific resins to remove nitrate from soil extracts.

Soil for these studies was collected from the humus ( $O_a$ ) layer of a forest stand dominated by oak at the Harvard Forest, Massachusetts. The soil samples were passed through a 2-mm sieve to remove large roots. Three samples of 88g field moist soil (50g dry weight) each were autoclaved and three samples were not autoclaved. Sterile solution of  $KNO_3$  were added to each to provide 50  $\mu g$   $NO_3^-$ -N/g soil. After 24 hours, all samples were extracted in 300 mL deionized water and suction filtered. The filtrates were stored frozen.

Aliquots of each sample were acidified to pH 2 to protonate organic acids before passing the solutions through Bio-Rad AG 1-X8 or the SR7 anion exchange resins to remove nitrate. The aliquots were then brought to pH 10 and allowed to sit open for 4 days to allow  $NH_3$  gas to diffuse out. However, ammonium remained in solution, so this approach was abandoned. Additional aliquots were again brought to pH 10 to deprotonate organic acids in solution and were then filtered through Bio-Rad AG 50W-X8 cation exchange resins that had been pre-treated with 1 N LiOH. The cation exchange resins were loaded with  $Li^+$  because this cation is more readily exchanged for  $NH_4^+$  compared to  $Na^+$  or other cations. Filtered solutions were analyzed for  $NH_4^+$ ,  $NO_3^-$ , and total N in a Lachat 8000 flow injection autoanalyzer.

Another necessarily preliminary investigation was to test the efficacy of persulfate digests of these solutions to measure DON. The extracts from the sterilized soil are highly colored, indicating the presence of high concentrations of organic acids that could overwhelm the persulfate oxidant or that could interfere with the pH-dependent chemistry of the persulfate digestion. The procedure of Bronk et al. (2000) was followed for persulfate digestion with aliquots of extract that were diluted by factors of 2, 4, 6, and 10.

Finally, we have been aware of a concern raised by other researchers (personal communications of unpublished results) of incomplete nitrate quantitation by flow-injection analyzers using the  $NH_4Cl$ -EDTA buffer method when high concentrations of soluble iron are present. Hence we have carried out experiments with nitrite and nitrate standard additions to our extracts, conducted measurements of soluble iron, and compared nitrate quantitation in aqueous extracts using both the Lachat autoanalyzer and an ion chromatograph.

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